

In vitro* Antitrypanosomal Activity of Fractions of Ethanol Extract from *Terminalia Cattapa* Leaf against *Trypanosomal Brucei Brucei

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Abstract

Trypanosomiasis is a tropical disease threatening human and animal health. Trypanosoma brucei brucei is unicellular parasites transmitted by the bite of tsetse fly and is the causative agent of sleeping sickness in humans and related diseases in animals. Over 25% of our common medicines contains some compounds obtained from plants. This research work is aimed at evaluating the invitro activity of fractionated ethanol extract of Terminalia cattapa leaf against Trypanosoma brucei brucei. Crude extract was obtained by cold maceration using absolute ethanol, followed by partial purification of the extract using column chromatography techniques. The test was performed in triplicates using a 96 well microtitre plate. Light microscope was used to view the parasite motility at magnification of × 400. Treatment with varying concentrations of pooled fractions F1, F2 and F3 (100, 50 and 25 mg/ml) resulted in reduction of number of motile parasites compared to the negative control loaded with parasites. In conclusion, fractions (F2 and F3) from ethanol extracts of Terminalia cattapa leaf have anti-trypanosoma activities against Trypanosoma brucei brucei in a dose depended manner with F3 being the most potent..

Keywords: Trypanosomiasis, *invitro*, Chromatography, Microscope, Motility

INTRODUCTION

Trypanosoma brucei rhodesiense and *T. brucei gambiense*, the causative agents of Human African Trypanosomiasis, are transmitted by tsetse flies. Trypanosomiasis is the name of several parasitic zoonotic diseases in both animals and humans, caused by protozoan haemoflagellate of the genus *Trypanosoma* which inhabit the blood plasma and various body tissues and fluids. In Nigeria, trypanosomiasis seems to be re-emerging as an important livestock disease, assuming major clinical importance in small ruminants and extending to previously designated tsetse-free zones (Ayodele, *et al.*, 2013). Trypanosomiasis is caused by trypanosoma species of various kinds: *Trypanosoma congolense*, *Trypanosoma vivax* and two subspecies of *Trypanosoma brucei* (*T. b. gambiense* causing the disease in West and Central Africa and *T. b. rhodesiense* causing the disease in East and Southern Africa) have a wide range of host in the wild and domesticated animals. The disease is transmitted by a bite of the vector – tsetse fly (*Glossina* species) (Archivio *et al.*, 2011).

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Sleeping sickness currently affects about half a million people in sub-Saharan Africa and an estimated 60 million people are at risk of contracting this disease, which is fatal if untreated. However, the currently available treatments are far from being ideal, chemotherapy is the most widely used means of controlling trypanosomiasis. The few registered trypanocides are often associated with severe side effects and require lengthy parenteral administration, lack efficacy and are unaffordable for most of the patients (Legros *et al.*, 2002).

The use of herbal preparation in traditional medicine has encouraged the sourcing of anti disease agents from indigenous vegetation (Atawodi *et al.*, 2003). *Terminalia catappa* Linn (family Combretaceae) also known as Indian almond is a plant species with good records of medicinal importance associated with its chemical constituents. *Terminalia catappa* L. is a monoecious tree with large leaves having the ability to protect against toxicants. Globally, fruit nutrients have beneficial health therapeutic and economic benefits (Venkatalakshmi and Brindha, 2016). In Nigeria, the most popularly used plant parts are commonly referred to as 'fruit' by some local folks. The seeds and leaf of the plant mostly contribute to a high percentage of waste in the environment without being utilized more effectively for the benefit of society. Indian almond is popularly used as a herbal treatment for management of diabetes mellitus (Divya *et al.*, 2018). It is also used as analgesic (Mohale *et al.*, 2008), for the alleviation of inflammation etc. Most often, the fruit parts (kernel endocarp and fleshy mesocarp) of *T. catappa* are consumed roasted, raw, and sun dried. Other plant parts such as the roots, barks, and leaf are used in the treatment of different ailments such as malaria, respiratory disorders (asthma) hypertension (Odugbemi, 2008). Several studies have reported that *T. catappa* can exert a multitude of effects such as anticancer and antioxidant antimicrobial, antidiabetic and hepatoprotective (Lee *et al.*, 2019).

The phytochemicals of this plant include tannins (puni-calagin, punicalin, terflavins A and B, tergalagin, ter-catain, chebulagic acid, geranin, granatin B, corilagin) Krishnaveni and Dhanalakshmi (Kumar *et al.*, 2018).

Previous studies on antitrypanosomal effect of crude extracts of *Terminalia cattapa* has indicated ethanol crude extract to be the most potent. This present work is aimed at fractionating ethanol extract and evaluating its *in vitro* antitrypanosomal effect against *trypanosoma brucei brucei*.

MATERIALS AND METHODS

Plant Materials and Authentication

Terminalia cattapa (leaf) was harvested fresh from a garden within Kaduna State University main campus and authenticated in the department of biological science, Kaduna State University and a Voucher No: KASU/BSH/043 was deposited.

Trypanosome Stabilates

The *trypanosoma brucei* strain (*Trypanosoma brucei brucei*) was obtained from the Nigerian Institute for Trypanosomiasis and Oncociasiasis Research (NITOR) Kaduna, Nigeria. The parasite was multiplied through animal passage.

Preparation of Ethanolic Extract of *Terminalia cattapa* Leaf

Ethanolic Extract of *Terminalia cattapa* Leaf was prepared according to the method described by Tabeshpour *et al.* (2017). The leaf of *Terminalia cattapa* was rinsed and dried for 3 weeks under room temperature and grinded into powder, 50g of the powdered leaf was soaked with 300ml ethanol in 500ml conical flask. The conical flask containing the mixture was capped with foil paper and shake vigorously for 48 hours. The mixture was filtered with a cheese cloth and then with filter paper, the filtrate was evaporated in water bath and dried extract was then weighed and fractionated using column chromatography.

Fractionation of Ethanol Extracts of *Terminalia cattapa*

Fractionation of ethanol extract of *T. cattapa* was done according to the method described by Abiodun *et al.* (2015). A slurry was prepared using silica gel and then loaded onto a chromatographic column (80 mm diameter × 450 mm height). Exactly (65) fractions (10 ml each) were obtained from hexane, ethanol and ethyl acetate partitions.

Hexane	Hexane :Ethyl acetate	Ethyl acetate:Ethanol	Ethanol
100%	50 : 50	50 : 50	100%

It is based on the adsorption of analytes in the crude extracts using a stationary phase (silica gel) and further separation of the mixture into discrete components. The rate of the movement of the components is expressed retention factor (Rf) which was calculated using the formular of Ahlam *et al.* (2019).

R f (retention factor) = the distance travelled by solute/ the distance travelled by the solvent

In Vitro* Test of Fractions of Ethanol Extract of *Terminalia cattapa* Leaf against *T. brucei brucei

In vitro test was performed as described by Hoet *et al.* (2004) infected blood was collected by cardiac puncture from a donor rat after the parasitemia have been monitored for 5 days and poured into EDTA bottle. The fractions was tested with thick blood films method (Atawodi *et al.*, 2003) to detect any motile trypanosomes which was done in triplicates in a 96 well microtitre plate (Flow laboratories Inc., McLean, Virginia 22101, USA) with 50 μ L of infected blood containing about 15-20 parasites per field as described by Nwodo *et al.* (2007) on determination of parasitaemia.

Parasitaemia was monitored after 60 minutes of incubation at 37 °C. About 0.5 μ L-1 μ L of test mixtures were observed every 2 minute under a microscope × 400 . A set of Positive Control (PC), Negative Control (NC) and Fraction Control (FC) were set up to stand for 4hrs with the infected blood containing the parasites. The PC contained Diminavetor (1.05 g of Diminazene diaceturate and 1.31 g Antipyrine), the NC contained only the infected blood suspended in Phosphate Buffer Saline Glucose (pH 7.2) and FC contained the fractions. Drop in motility of the parasites in fractions treated blood compared to that of parasite-loaded control blood without fractions was taken as a measure of antitrypanosomal activity (Nagagi *et al.*, 2016).

Statistical Analysis

All tests were carried out in triplicate and results were expressed as mean ± standard deviation. Significant differences were analyzed between groups using one-way analysis of

variance (ANOVA) followed by Turkey- Kramer Multiple Test (graphpad Instat 3.10 software) with statistical significance set at $p < 0.05$.

RESULTS AND DISCUSSION

The adsorption of solute molecules to the column plate occurs in a reversible manner and movement was identified by band formation, the fractions with same RF values were pooled together resulting to three (3) fractions (table 1).

Table 1: Retention Factor of Fractions of *Terminalia cattapa*

Fractions	Retention Factor (cm)
F1	0.644
F2	0.731
F3	0.556

There was no parasite motility in the drug treated plate. F1, F2, and F3 are pooled fractions of *T. cattapa*. A significant difference was observed in F2 and F3 when compared to F1 and negative control. At lower concentrations of (25 mg/ml) of F1, parasites were motile as observed in the negative control plate but there was reduction in the viable parasites in plates treated with fractions F2 and F3 at different concentrations when compared with the negative control loaded with parasites, this is in conformity with the findings of Abiodun *et al.* (2012) on antitrypanosomal activity of *Garcinia kola* seed extract which resulted in significant suppression and total clearance of the parasitemia.

The positive control treated with diminazene aceturate (3.5mg/ml) standard drug, cleared the parasites after an hour and remain constant even after two hours of incubation with the drug, by totally inhibiting motility of parasites. This agrees with the report of Taylor and Authie (2004) on the pathogenesis of animal trypanosomes.

In this study, the highest trypanocidal activity was recorded in fraction 3 at 100 mg/ml concentration, where the highest parasite motility was observed. This conforms with the work of Bashir *et al.* (2015) who reported that twenty-three West African plants inhibit Trypanosome parasite motility within minutes. For the negative control (parasite with glucose phosphate-buffered solution) and F1 (10 mg/ml) no seizure of parasite motility was observed.

Therefore, this study's plant extract activity may be attributed to the phytoconstituents such as flavonoid, alkaloids, tannins present in the plant extracts, which had been implicated in trypanosomal activities (Nagappa, 2003). Tannins have been reported to form complexes with proteins through non-specific forces therefore, resulting in decreased parasite motility (Taylor, 2000).

Furthermore, natural products contain structures that generate radicals, which can cause oxidative damage to trypanothione reductase, which is extremely sensitive to redox balance changes (Nibret *et al.*, 2010). Metals chelation which in turn inhibit lipid peroxidation in trypanosomes by flavonoids and flavonoid-derived products have also been suggested to contribute to antitrypanosomal activity (Harbourne and Williams, 2000).

Table 5: Number of Motile *T.brucei brucei* Parasite in Micro titre Plates Treated with Fractionated Ethanolic Extract of *T. cattapa*

Fractions	Number of Motile Parasites 100 (mg/ml)	Number of Motile Parasites 50 (mg/ml)	Number of Motile Parasites 25 (mg/ml)
F1	23.75 ± 0.96 ^a	26.72 ± 0.75 ^a	30.31 ± 1.32 ^a
F2	6.50 ± 0.58 ^b	12.51 ± 0.9 ^b	15.16 ± 1.21 ^b
F3	5.50 ± 0.58 ^b	10.45 ± 1.12 ^b	12.36 ± 0.57 ^c
Negative control	30.75 ± 0.96 ^c	30.75 ± 0.96 ^c	30.75 ± 0.96 ^a
Standard drug (3.5mg/ml)	0.00	0.00	0.00

values are expressed as mean ± standard deviation. Values with different superscripts down the column are significantly different ($p < 0.05$).

CONCLUSION

T. catappa has antitrypanosomal activity against *T. brucei brucei* as shown by the decrease in the number of motile parasites in the treated plates. The antitrypanosomal activity of the different fractions of *T.catappa* was dose depended and attributed to the presence of phytochemicals which include tannins and flavonoids. There should be further purification of *T. catappa* bioactive components to evaluate the actual active component responsible for the anti-trypanosomal activities.

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